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QUANTITATIVE ANALYSIS OF PAPAVER BRACTEATUM[2/]

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1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories.

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QUANTITATIVE ANALYSIS OF PAPAVER BRACTEATUM

The procedures which we are now following for the quantitative analysis of P. bracteatum for thebaine and isothebaine are as follows:

Plant material is dried to constant weight at 105° in a drying oven. Larger roots are cut into pieces 1 cm long prior to drying. Plant material is then ground by mortar and pestle to pass through a 0.5 mm sieve. The ground plant material is mixed thoroughly, with the aid of a spatula, before a sample is selected for analysis.

A 100 mg sample is weighed and the sample is extracted at room temperature twice with 30 ml portions of 3% acetic acid. The mixture is stirred 20 minutes with a magnetic stirrer during each extraction. Extracts are separated by filtration, the marc washed with an additional 10 ml of 3% acetic acid after the second extraction, and the extracts and washing are combined.

This solution is extracted three times with 20 ml portions of hexane. The pH of the aqueous solution is raised to 9 by the addition of sodium carbonate. The resulting mixture is extracted three times with 40 ml portions of ethyl ether. The combined ether extracts are washed once with 30 ml of water. The ether is evaporated, in vacuo, until the volume is about 10 ml. The ether solution is transferred to a 25 ml round bottom flask for final drying. The weight of the residue is then determined. This residue may be used for GLC or TLC studies.

GLC studies are being conducted with a Varian model 1200 gas chromatograph equipped with a hydrogen flame detector. A seven foot 1/8 inch stainless steel column packed with 3% OV-17 on 100/200 mesh Gas Chrom Q is being utilized. The carrier gas is nitrogen at a flow rate of 40 or 50 ml per min. Programmed and isothermal procedures have been utilized. The attenuation is set at 64×10^{-11} allowing the detection of .01 µg. of alkaloid. Sensitivity can be increased further, if needed. Routine analyses are being run isothermally at a column temperature of 260°. The inlet and detector are kept at 290°. A one or two µl. sample was normally injected. Peak area measurements were made using the method of peak height times width at half-height. The peak area of each alkaloid was compared with the peak area of the internal standard. Using the appropriate correction factors, the alkaloid concentration was then determined.

The new internal standard adopted is cholesterol acetate (1.0). Relative retention times of interest are thebaine (0.60), isothebaine (0.84) and cholesterol acetate (1.0). The internal standard is dissolved in n-butanol and kept in a tightly sealed container at room temperature. n-Butanol was selected as a solvent because its high boiling point would minimize evaporation and changes in concentration. Fresh solutions were made every two months, although no changes in concentration were detected. Calibration curves between the internal standard, cholesterol acetate, and the alkaloids, thebaine and isothebaine were made and checked every week. A standard sample of thebaine or P. bracteatum extract was run every day that analyses were made.

A series of drying experiments were conducted. Cut root pieces dry to constant weight in 12 hours at 105° and in 24 hours at 60°. Leaves dry even more rapidly. All dried plant parts pick up moisture if exposed to the air. Thus, samples should be freshly dried or stored in a dry, air-tight container, prior to analysis.

No difference in analysis could be detected between drying samples at 60° and 105°, if dried to constant weight. Thus, we now dry the samples overnight at 105°.